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# PELLETS for CONTROLLING ORGANISMS in MAPLE TREE TAPHOLES



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# THE EFFICIENCY AND PRACTICABILITY OF DIFFERENT TYPES OF PARAFORMALDEHYDE PELLETS FOR CONTROLLING MICROBIAL GROWTH IN MAPLE TREE TAPHOLES<sup>1</sup>

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MICROORGANISMS are the primary cause of the premature decline and stoppage of sap flow from maple tree tapholes (2 and 5). Sheneman *et al.* (5) demonstrated this by use of a sanitary tapping procedure combined with a closed system for collecting sap to protect against contamination and by using microbial inhibitors. There was a highly significant correlation between microbial counts in tapholes and sap yield from these tapholes. The earlier in the season that significant numbers of microorganisms were observed in sap, the lower the sap yield. Tapping in advance of the season, high initial microbial populations and warm weather early in the sap season all resulted in increased microbial activities and low sap yields.

The actual mechanisms by which microorganisms stop the flow of sap has not been clearly elucidated. Ching and Mericle (1) reported that the stoppage resulted from the "combined effects of a bacterial invasion of the living tissues via pits along the cell walls, and an actual vessel blockage by microorganisms and/or gummy plugs produced by these microorganisms." They also concluded that yeasts and molds probably are not responsible for premature stoppage of

<sup>1</sup>A report of work done under contract with the U. S. Department of Agriculture and authorized by the Research and Marketing Act of 1946. The contract is being supervised by the Eastern Utilization Research and Development Division of the Agricultural Research Service.

sap flow. However, Sheneman *et al.* (5) demonstrated that yeasts and molds as well as bacteria would stop sap flow when inoculated into tapholes; and suggested that the effect of microorganisms may be purely physical in nature.

Since many unrelated microorganisms are capable of causing a reduction and stoppage of maple sap flow, any agent used to prevent their development would have to have a broad antimicrobial spectrum. Ideally, such an agent should have the following characteristics:

- (a) high antimicrobial activity in extremely low concentrations.
- (b) adaptable to incorporation in a pellet with a slow disintegration rate, to provide a low but adequate level in the taphole throughout the maple season.
- (c) have no effect on the flavor or color of the syrup produced from maple sap.
- (d) completely non-toxic.
- (e) completely volatilized or destroyed during the boiling of the sap to produce syrup.

Sheneman *et al.* (5) tested a number of compounds for their efficiency in controlling microorganisms in tapholes. These included aureomycin, hypochlorite and Roccal<sup>2</sup> rinses, "Sterilite" powder<sup>3</sup> and cotton treated with "O-Silver" aqueous,<sup>4</sup> and pellets of sorbic acid, paraformaldehyde, and mercuric iodide. Of these treatments, only the paraformaldehyde and mercuric iodide pellets and the "Sterilite" powder proved effective and only paraformaldehyde appeared promising for commercial use.

This study was designed to test the efficiency of various types of paraformaldehyde pellets in inhibiting microbial growth in maple tree tapholes; and to determine the extent of formaldehyde residue which may be found in syrups produced from sap collected from tapholes containing paraformaldehyde pellets of different formulations and shapes. From these data, it was hoped that a specific paraformaldehyde pellet formulation might be recommended.

## MATERIALS AND METHODS

**Effect of formaldehyde on representative cultures of molds, yeasts and bacteria isolated from maple tree tapholes:** The cultures of

<sup>2</sup> Winthrop-Stearns, Inc., New York 18, New York.

<sup>3</sup> Colloidal carbon coated with colloidal silver supplied by the Shellmar-Betner Flexible Packaging Division of Continental Can Co., Inc., Mount Vernon, Ohio.

<sup>4</sup> A suspension of oligodynamic silver supplied by the Chloramine Co., 54 West 16th Street, New York 11, New York.

microorganisms used in these studies were from the collection studied by Sheneman and Costilow (4). All cultures were grown on Tryptone glucose extract agar<sup>5</sup> (TGEA) and the cells washed from slants with 10 ml. sterile saline to prepare initial suspensions. This resulted in suspensions containing about  $10^9$  cells/ml. with the bacteria and between  $10^7$  and  $10^8$ /ml. with the molds and yeasts. These initial suspensions were diluted 10X, 100X, and 10,000X for inoculation into test media.

Two series of tests were run. In one series, 0.1 ml. of each of the final suspensions was inoculated into broths containing 0.5 percent peptone and 3 percent dextrose, and formaldehyde levels of 0, 1, 10, 25, 50 and 100 p.p.m. In the second test series, 1 ml. of each was placed in petri dishes and the plates poured with TGEA containing formaldehyde levels of 0, 20, 30, 50, 100, 150 p.p.m. All test cultures were incubated for 7 days at room temperature ( $22 \pm 2$  C.) and examined for growth.

**Preparation of paraformaldehyde pellets:** Paraformaldehyde in agar pellets similar to those used by Sheneman *et al.* (5) was prepared in this laboratory. These were made by preparing a slurry containing equal weights of paraformaldehyde and hot melted agar.<sup>6</sup> This slurry was maintained at a temperature above 50 C., and stirred continuously until it was pulled by vacuum into 4 ft. lengths of 7mm. ID pyrex tubing. After solidification, the mixture was pushed out of the tubing and cut into lengths containing the desired weight of paraformaldehyde.

Dr. Wilhelm Hurka, Arcana K. G., Lieserbrücke, Kärnten, Austria, prepared machine made paraformaldehyde pellets (tablets), 9 mm. in diameter, for the tests. Solution rate tests were run with 400 mg. paraformaldehyde pellets of the following types: (1) two lots with no binder, having 10-11 and 14-17 kg. test strength,<sup>7</sup> respectively; (2) with 0.5, 1.0, 2.0, and 5 percent case in binder with test strengths of 10-11 kg; and (3) with 0.5, 1.0, 2.0, and 5.0 percent gum arabic binder with test strengths of 14-17 kg. The 200 mg. pressurized pellets used in tapholes during the 1960 season had a test strength of 14-15 kg. without binder and 10-12 kg. when either 0.5 or 1.0 percent gum arabic was included. The 400 mg. pellets had a strength of 15-16 kg.,

<sup>5</sup> Difco Laboratories, Inc., Detroit, Mich.

<sup>6</sup> 2.5 or 5.0 g. agar dissolved in 100 ml. water.

<sup>7</sup> Test strength refers to the crushing force required for disintegration as measured by the Stokes pellet tester, F. J. Stokes Machine Co., 5932 Tabor Rd., Philadelphia, Pa.

either with or without binder. The 400 mg. pellets used in 1961 were of the same type as those used in 1960.

The various pellet types used are illustrated in Fig. 1.

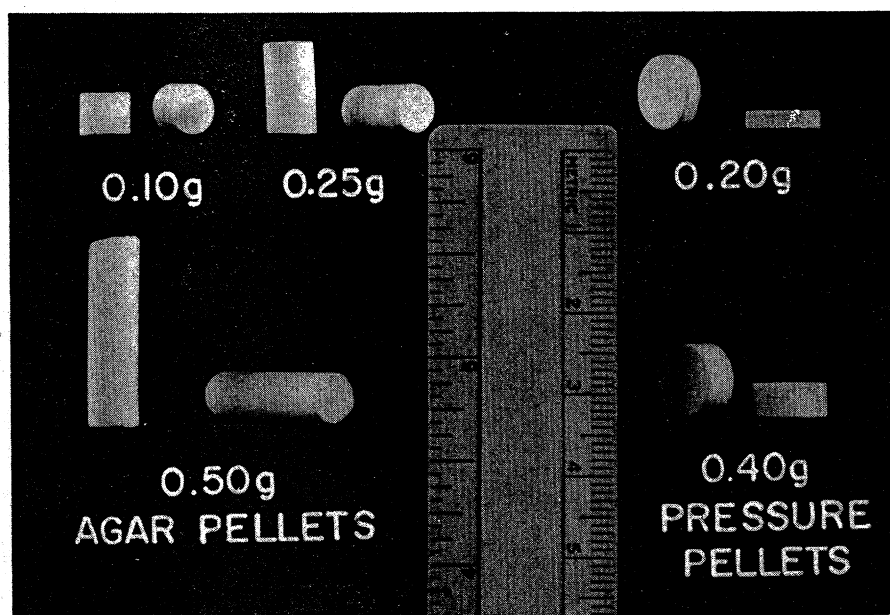


Fig. 1. Various paraformaldehyde pellet types tested. All pellets to the left of the rules are agar type; all to the right are pressure pellets.

**Design of maple tree taphole treatment experiments:** A carefully controlled series of experiments involved the treatment of 50 trees in 1959 (one was destroyed by a windstorm), 60 trees in 1960, and 100 in 1961 (see Table 1). These trees were all in the Baker woodlot on the Michigan State University campus and were selected at random from a larger group chosen on the basis of previous records for high yield. The trees were all numbered and randomized for treatment. For the purpose of this paper, treatment will refer to the insertion of a paraformaldehyde pellet in a taphole.

As indicated in Table 1, three tappings were made per tree in 1959 and 1960 and two per tree in 1961. The tappings were made in the West quadrant of the tree in 1959, the East quadrant in 1960, and in the South quadrant in 1961. The relative position of the treatments in the quadrant was randomized each year. Tappings were made to a depth of 3 inches.

The amount of maple sap from each taphole was weighed to the nearest 0.1 lb.

An additional experiment was conducted in a separate sugar bush (Sanford woodlot) on the Michigan State University campus in 1961. Two hundred trees were tapped with two tapholes per tree; one serving as a control and the other treated with a 400 mg. paraformaldehyde pressure pellet. The sap was collected separately from the control and treated tapholes, and the total yield from each measured on a volume basis.

Also, during the 1961 season, the pressure pellet of 0.4 g. paraformaldehyde was placed in 500 tapholes in a sugar bush on a farm in Eaton County, Michigan, while an adjacent sugar bush with 500 tapholes was not treated. Yield records were maintained by the farmer. Records for several preceding years demonstrated that the average sap yields from the two woodlots were similar.

**Collection of maple sap and syrup samples and determination of microbial populations and formaldehyde concentrations:** Sap samples from individual trees for determination of microbial populations and formaldehyde concentrations were collected by hanging sterile ½-pint milk bottles from the spiles. A few ml. of sap were allowed to drip into the bottle and the bottle recapped.

Total microbial counts were run on the sap samples by plating with TGEA. Formaldehyde concentrations were estimated by the Hantzsch reaction method of Nash (3). The clear maple sap does not cause any significant interference in the analytical method so that analysis can be made on the sap "as is" without dilution or distillation.

Samples of pooled saps representing individual treatments and samples of syrups, produced from these saps by boiling in a fire arch evaporator, were analyzed for formaldehyde. It was necessary to distill a small fraction (2-3 ml. of 20-25 ml.) of the syrup sample prior to running the Hantzsch reaction to reduce the development of interfering colors. A number of syrup samples from control saps were run to determine the extent of the interfering reaction, and the treated samples corrected accordingly. This average correction was equivalent to 0.90 p.p.m. formaldehyde.

Formaldehyde concentration in pellets was determined by the same technique as described above after dissolving the pellets in 100 ml. of slightly alkaline distilled water.

## RESULTS

**Effect of formaldehyde on representative species of bacteria, yeasts, and molds:** Microorganisms vary greatly with respect to their sensitivity to formaldehyde. The data in Fig. 2 reflect the highest levels of formaldehyde at which growth was obtained and the lowest levels at which all tests were negative. In general, the bacteria were the most sensitive of the organisms tested, being inhibited completely by levels of 30 p.p.m. or less. The yeasts, *Rhodotorula glutinis* and *Trichosporon pullulans*, were of intermediate resistance; and the molds tolerated relatively high concentrations (over 100 p.p.m.). Fortunately, the species found to predominate in maple tapholes, *Pseudomonas geniculata* (4) was one of the more sensitive species. It was inhibited completely by 30 p.p.m., even with an inoculation of  $10^8$  cells per ml., and was inhibited greatly by 20 p.p.m. when lower inoculation levels were used.

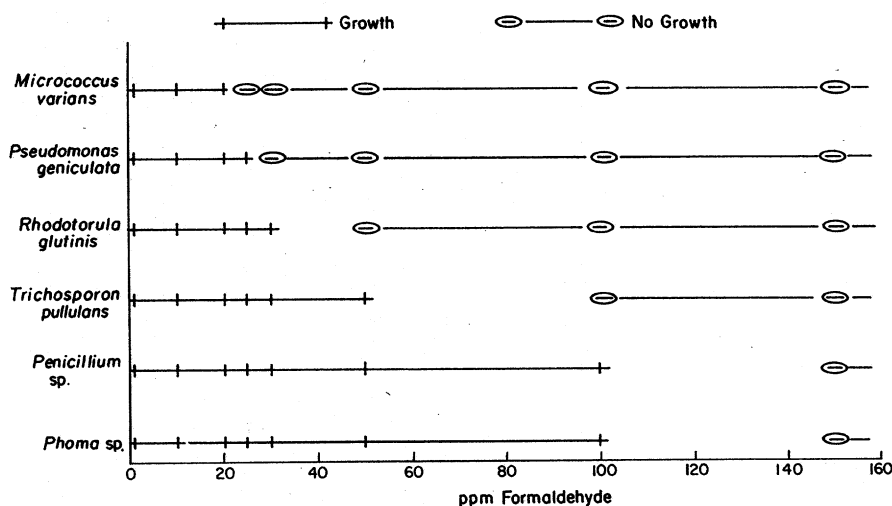


Fig. 2. Effectiveness of formaldehyde in inhibiting growth of various microorganisms. Two growth tests were run, one in liquid and one in solid media. Growth is indicated as positive if the organism grew in either medium.

**Control of microorganisms in maple tree tapholes:** As predicted from the preliminary data of Sheneman *et al.* (5), paraformaldehyde pellets placed in maple tree tapholes greatly inhibited microbial development. There was no measureable difference in microbial control between the two pellet types used in 1959. In 1960, the 0.1 g. para-

formaldehyde in agar formulation appeared to be slightly less effective than the other types used; and, in 1961, a small difference was noted in favor of the agar pellet over the pressure pellet. However, small differences in microbial populations are of doubtful significance, so the data for all paraformaldehyde treatments were included in the average for each season.

Since uniform control throughout the season is desired and averages of populations do not reflect this, the percentage of maple sap samples examined having populations over  $10^4$  per ml. was calculated for each date that samples were run. In most instances, there were between 40 and 60 control and 75 and 110 treated samples examined on each date. The population level of  $10^4$  per ml. was selected because it was noted by Sheneman *et al.* (5) to "represent a condition of incipient decline in yield from the taphole" of maple trees, and was used by them in the statistical analysis of the correlation of microbial populations and sap yield.

The paraformaldehyde treatments were effective in inhibiting the development of microorganisms in the taphole during all three seasons (Fig. 3). Less than 30 percent of the sap samples from the treated tappings had populations over  $10^4$ /ml. when approximately 90 percent of the comparable control samples had counts in excess of  $10^4$ /ml. The influence of seasonal temperatures is reflected quite accurately in these data. In 1959 and 1960, the months of February and March were quite cold and very little sap flowed until late in the season, while in 1961 temperatures were quite high for some periods early in the season. Similarly, microbial development was greatly delayed even in the control tappings in 1959 and 1960 as compared to 1961.

**Effectiveness of paraformaldehyde pellets of different formulations in increasing maple sap yield:** The effect of paraformaldehyde treatment on sap yields for the three sap seasons correlates closely with the microbial patterns. In the cold season of 1959, there was very little microbial growth in the control tapholes until after April 1, and the pellet treated tapholes produced highly significant but relatively low increases (14 to 20 percent) in sap yield (Table 1). In the very cold season of 1960, it was late in April before any appreciable microbial populations were observed in the maple sap, and paraformaldehyde treatments failed to have any significant effect on sap yield.

The warm weather occurring late in February and early in March, 1961, resulted in the most dramatic effect of paraformaldehyde treat-

ments on sap yield. Treatment with the 5 percent agar pellet containing 0.5 g. paraformaldehyde increased sap yield by 77 percent while the 0.4 g. pressure pellet increased the yield by only 46 percent. These were both statistically significant increases in yield at the 1 percent probability level. The greater yield observed with the agar pellet than with the pressure pellet was also highly significant.

The significant difference in yield from tapholes treated with the two kinds of pellets in 1961 is of particular interest. This correlates with an observed difference in microbial populations obtained with these two treatments which was of doubtful significance due to the large error possible in microbial counts. However, the yield data indicates that the difference observed was probably real.

The increases in yield due to the paraformaldehyde treatments in 1961 were not the result of a longer sap running time alone, since the differences in yields were significant when the April yields were excluded. All tapholes were still flowing at the end of March, but the rate of flow of the average control had been greatly reduced.

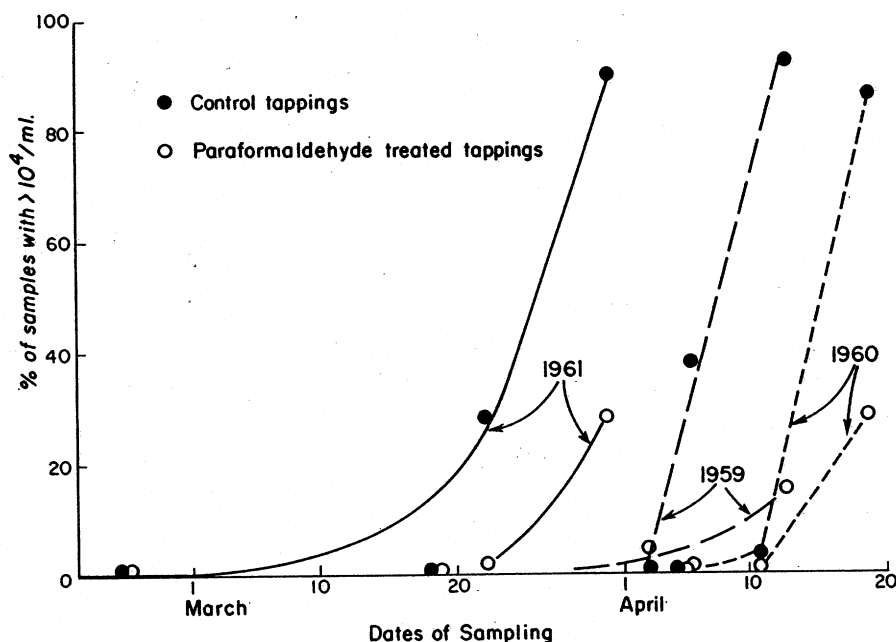


Fig. 3. Effectiveness of paraformaldehyde pellets in controlling microbial growth in tapholes of maple trees. Total microbial counts were run on individual sap samples at the sampling dates indicated, and the percentage of the individual sap samples having populations in excess of  $10^4$ /ml. calculated and plotted.

**TABLE 1—Yields of maple sap from various paraformaldehyde treatments**

Season	Paraformaldehyde treatment	Number of tap-holes	Average yield of sap per taphole		Increase in yield	
			Pounds	Gallons	Pounds	Percent
1959	Control.....	49	116	13.9	....	....
	0.25g in 5% agar.....	49	133	15.9	17.0	14.4**
	0.50g in 5% agar.....	49	139	16.7	23.5	20.3**
1960	Control.....	12	98	11.7	...	...
	0.10g in 5.0% agar.....	12	95	11.3	-3.0	-3.1
	0.25g in 5.0% agar.....	12	102	12.2	4	4.1
	Control.....	12	99	11.8	...	...
	0.10g in 2.5% agar.....	12	103	12.2	4	4.0
	0.25g in 2.5% agar.....	12	97	11.5	-2	-2.0
	Control.....	12	87	10.4	...	...
	0.20g pressure pellet.....	12	93	11.1	6	7.0
	0.40g pressure pellet.....	12	81	9.8	-6	-7.0
	Control.....	12	92	11.0	...	...
	0.20g+0.2% gum arabic .	12	92	11.0	0	0
	0.40g+0.2% gum arabic .	12	89	10.6	-3	-3.3
	Control.....	12	97	11.5	...	...
	0.20g+1.0% gum arabic .	12	99	11.8	2	2.1
	0.40g+1.0% gum arabic .	12	79	9.4	-18	-18.6
	All controls.....	60	94.6	11.28	...	...
	All treated.....	120	93.0	11.00	-1.6	-1.8
	Control.....	50	118	14.1	...	...
	0.5g in 5% agar.....	50	209	25.0	91	77.2**
	Control.....	50	115	13.7	...	...
	0.4g pressure pellet.....	50	168	20.1	53	46.1**
	All controls.....	100	116.5	13.9	...	...
	All treated.....	100	188.5	22.65	72	61.8**
1961	Control.....	50	118	14.1	...	...
	0.5g in 5% agar.....	50	209	25.0	91	77.2**
	Control.....	50	115	13.7	...	...
	0.4g pressure pellet.....	50	168	20.1	53	46.1**
	All controls.....	100	116.5	13.9	...	...
	All treated.....	100	188.5	22.65	72	61.8**

\*\* Highly significant increase in yield.

The two field trials of 1961 yielded data similar to the above. The average sap yield from the 200 treated tapholes in the Sanford woodlot located on the Michigan State University campus was 15.8 gal., while the corresponding controls yielded an average of 10.2 gal., an average increase of about 55 percent resulting from paraformaldehyde treatment.

The 500 treated tapholes on the Eaton County farm had produced an average of 22.5 gal. by March 25 and 27.1 gal. by April 5, while the

500 control tapholes had produced averages of 14.6 and 15.1 gal. for the same two periods, respectively. This amounts to increases in yield of about 54 percent by March 25 and 80 percent by April 5 resulting from paraformaldehyde treatment. The increases in yield observed in both of these field trials were statistically highly significant.

**Effect of paraformaldehyde treatment of tapholes on the quality of the syrup made from the sap:** Finished maple syrups from saps from treated and control tapholes were graded for quality according to the U. S. Standards for Maple Syrup (U. S. Agricultural Marketing Service, 1940) using permanent glass color standards. Syrups of high quality were obtained from saps from both treated and control tappings initially in both seasons (Table 2). However, the quality of the syrup made from saps from treated tappings remained at a higher level than those from corresponding controls throughout the latter part of the two seasons. No deleterious effect was noted in any syrups obtained from treated tapholes.

**TABLE 2—U. S. grades of maple syrups from control and treated tappings**

Date of sap flow		1960 season		1961 season	
		Control	Treated	Control	Treated
March	4....	(a)	...	AA	AA
	14....	...	...	AA	AA
	16....	...	...	AA	AA
	21....	...	...	AA	AA
	22....	...	...	A	AA
	23....	...	...	A	AA
	25....	...	...	B	A
	28....	AA	AA	...	...
	31....	AA	AA	...	...
April	2....	A	AA	...	...
	8....	A	AA	Unc.(b)	B
	11....	B	A	...	...
	13....	B	A	...	...

(a) No syrup made.

(b) Unclassified, refers to grade given very dark syrups.

**Formaldehyde concentrations occurring in maple sap from paraformaldehyde treated tapholes and in syrups made from this sap:** The literature on the chemical and physical properties of paraformaldehyde and formaldehyde has been comprehensively reviewed and sum-

marized by Walker (7). Paraformaldehyde dissolves slowly in cold water to yield solutions identical to those prepared by dissolving gaseous formaldehyde. Formaldehyde is completely monomeric in solutions containing 2 percent or less in the form of the monohydrate which is methylene glycol. The hydrate is probably decomposed at the boiling point of water, but the formaldehyde concentration in solution does not change rapidly during boiling. On distillation at atmospheric pressure, solutions of approximately 8 percent formaldehyde behave like constant boiling mixtures; with less concentrated solutions, the residue becomes more dilute, and with solutions over 8 percent the residue becomes richer in formaldehyde.

Formaldehyde is not a permissive food additive. Therefore, it was necessary to investigate the possibility of a residue occurring in maple syrups when various types of pellets were used in the tapholes. Fortunately, the formaldehyde concentrations were quite low in the saps flowing from treated tapholes (Table 3). In 1959, there were only four samples of 437 examined with more than 10 p.p.m. formaldehyde; and 87 percent of 221 sap samples from tapholes treated with

**TABLE 3—Relative formaldehyde concentrations observed in individual sap samples from tapholes with various treatments**

Season	Paraformaldehyde treatment	Number of samplings	Number of samples	Percent of samples with concentrations of:			
				<1 p.p.m.	1-5 p.p.m.	5-10 p.p.m.	>10 p.p.m.
1959	0.25g in 5.0% agar.....	6	221	87.0	11.1	1.4	0.5
	0.50g in 5.0% agar.....	6	216	73.1	24.6	0.9	1.4
	Pooled sap samples:						
	0.25g in 5% agar.....	4	4	100.0	0.0	0.0	0.0
	0.50g in 5% agar.....	4	4	75.0	25.0	0.0	0.0
1960	0.10g in 2.5% agar.....	4	48	83.3	16.7	0.0	0.0
	0.10g in 5.0% agar.....	4	46	78.5	17.0	4.5	0.0
	0.25g in 2.5% agar.....	4	48	56.2	22.9	20.9	0.0
	0.25g in 5.0% agar.....	4	47	60.4	33.5	6.1	0.0
	0.2g pressure pellet.....	4	45	65.0	30.7	4.3	0.0
	0.4g pressure pellet.....	4	44	58.7	15.8	16.0	9.5
	0.2g+0.2% gum arabic ..	4	45	66.7	22.9	8.3	2.1
	0.4g+0.2% gum arabic ..	4	44	63.6	15.2	21.2	0.0
	0.2g+1.0% gum arabic ..	4	43	63.9	24.5	11.6	0.0
	0.4g+1.0% gum arabic ..	4	42	49.0	18.0	21.0	12.0
	Pooled sap samples:						
1961	(a) 0.5g in 5% agar....	5	5	40.0	60.0	0.0	0.0
	(b) 0.4g pellets.....	5	5	100.0	0.0	0.0	0.0
	(c) Both (a) and (b)....	2	2	100.0	0.0	0.0	0.0
	TOTALS.....	..	901	72.0	20.0	6.5	1.5

the 0.25 g. paraformaldehyde in 5.0 percent agar pellets contained less than 1 p.p.m. Pooled sap samples from the lower treatment all contained less than 1 p.p.m., and all but one pooled sample from the higher treatment was less than 1 p.p.m., and this sample contained only 1.2-1.4 p.p.m.

The 1960 data show considerable variation in the formaldehyde concentrations observed in sap. In general, the range of formaldehyde concentrations was lower with the smaller pellet of each type than with the larger pellet. Thus, the 0.1 g. paraformaldehyde in agar pellets resulted in lower formaldehyde concentrations in the sap than did the comparable 0.25 g. pellets; and the 0.2 g. pressure pellets of pure paraformaldehyde resulted in lower levels than did the 0.4 g. pellets.

The treatment with 0.4 g. pressure pellet either with or without gum arabic appeared to yield the highest formaldehyde levels in the sap. More than 25 percent of the samples from the three treatments with this type pellet had over 5 p.p.m. formaldehyde, and an appreciable number of the samples had concentrations over 10 p.p.m. The 0.25 g. paraformaldehyde pellets in 5 percent agar or the 0.1 g. paraformaldehyde in either 2.5 or 5.0 percent agar pellets appeared the most satisfactory from the standpoint of the amount of residual formaldehyde in the sap.

No syrup samples made from maple sap from paraformaldehyde-treated tapholes were found to contain more than 1.6 p.p.m. formaldehyde, and the three samples reported to have this concentration and the two having 1.3 p.p.m. were all prepared from a single day's sap collection in 1960 (Table 4). There was a single sample in 1959 which was found to have 1.2 p.p.m. and another sample in 1961 with 1.0 p.p.m. formaldehyde, and both of these were prepared from saps collected from tapholes treated with either 0.4 or 0.5 g. paraformaldehyde pellets. The remaining 38 of the 45 syrups prepared and tested contained less than 1 p.p.m. formaldehyde.

As expected, the concentration of formaldehyde in syrup was in practically all instances lower than that in the sap before concentration. Saps with 1 to 1.5 p.p.m. formaldehyde yielded syrups with less than 1 p.p.m. However, when syrup was made from sap to which 7.5 p.p.m. formaldehyde had been added, a residue of 5.3 p.p.m. was observed. Therefore, it is absolutely essential that the formaldehyde concentration be prevented from building up in maple sap to prevent the occurrence of a significant residue in the syrup.

**TABLE 4—Relative formaldehyde concentrations observed in syrups made from saps collected from paraformaldehyde treated tapholes**

Season	Paraformaldehyde treatment	Number of syrup samples	Number of samples with concentrations of:		
			<1 p.p.m.	1-1.5 p.p.m.	1.5-2.0 p.p.m.
1959	0.25g in 5.0% agar.....	5	5	0	0
	0.50g in 5.0% agar.....	6	5	1 (1.2 p.p.m.)	0
1960	0.10g in 2.5% agar.....	3	3	0	0
	0.10g in 5.0% agar.....	3	3	0	0
	0.25g in 2.5% agar.....	3	3	0	0
	0.25g in 5.0% agar.....	3	2	0	1 (1.6 p.p.m.)(a)
	0.20g pressure pellet.....	2	2	0	0
	0.40g pressure pellet.....	2	1	0	1 (1.6 p.p.m.)(a)
	0.20g +0.2% gum arabic ..	2	2	0	0
	0.40g +0.2% gum arabic ..	3	2	1 (1.3 p.p.m.)(a)	0
	0.20g +1.0% gum arabic ..	2	1	1 (1.3 p.p.m.)(a)	0
	0.40g +1.0% gum arabic ..	2	1	0	1 (1.6 p.p.m.)(a)
	0.50g in 5.0% agar.....	3	3	0	0
	0.40g pressure pellet.....	4	4	0	0
1961	Combined treatments.....	2	1	1 (1.0 p.p.m.)	0
	TOTALS.....	45	38 (84%)	4 (9%)	3 (7%)

(a) These samples were all prepared from a single day's sap collection.

**Rates of solution of paraformaldehyde in water and rates of loss during dry storage:** It is important that any paraformaldehyde pellet used in maple tree tapholes hold its structural integrity throughout the maple season and go into solution at a rate slow enough to prevent the occurrence of high formaldehyde levels in the maple sap. Also, it should be possible to store the pellets for at least a few months without losing their effectiveness. Therefore, experiments were conducted to test various pellet types for these characteristics.

Individual pellets were stored in 400 ml. of distilled water (pH 7.0) in amber glass bottles at 4 C. The pellet type had a pronounced effect on the solution rate in water (Fig. 4). After 1 day, the water with the agar pellets had higher formaldehyde concentrations than did the water covering pressure pellets. However, after 1 or 2 days, the rate of solution from the agar pellets was less than that from the pressure pellets without binder. After 14 days in water, only 32-34 percent of the paraformaldehyde had been dissolved out of the agar pellets, while 60-63 percent of that in the pressure pellets was in solution. Pellets prepared with 2.5 percent agar were comparable to those prepared with 5.0 percent agar. The solution rates of pressure

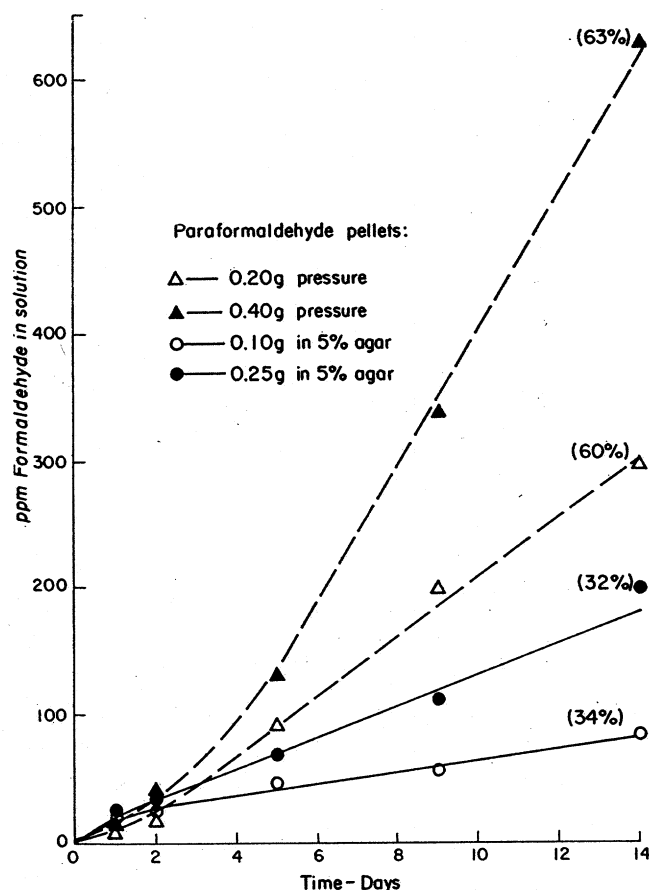


Fig. 4. Solution rates of different paraformaldehyde pellets in 400 ml. of water (pH 7.0) at 4 C. The figures given in parentheses reflect the percentages of the total amounts of paraformaldehyde contained in the pellets that were in solution after 14 days.

pellets containing either 0.2 or 1.0 percent gum arabic were somewhat lower than that of the pressure pellets, but still higher than the rates of the agar pellets.

All the agar pellets and the pressure pellets without binder maintained their structural shape throughout the test period, although the latter appeared somewhat crumbly around the edges when the test was terminated. However, pressure pellets containing gum arabic began crumbling rapidly before the end of 7 days in the water. Pellets

prepared with either 0.5, 1.0, 2.0 or 5 percent casein as a binder crumbled in water more rapidly than those containing comparable amounts of gum arabic.

Solution rates in water at 4 C. flowing through simulated tapholes at an average rate of 66 ml. per hour (20 drops per min.) were observed over a 7-day period. The simulated tapholes were made of glass and allowed for a hold-up volume of about 5 ml. Thus, there was a complete change of the water over the pellet in about 5 minutes. The water that flowed over the 5 percent agar pellets with 0.25 g. paraformaldehyde contained an average of 5.9 p.p.m. formaldehyde, while that which flowed over the 0.1 g. pellet contained 3.6 p.p.m. formaldehyde. The 0.4 and 0.2 g. pressure pellets resulted in an average of 7.9 and 3.5 p.p.m. formaldehyde, respectively, in the water; and the 0.4 and 0.2 g. pellets with 1 percent gum arabic contributed an average of 4.6 and 2.7 p.p.m., respectively. None of the pellets were observed to crumble during the test period.

Observations were made of pellets under actual field conditions in 1960. Many of the pellets were removed from the tapholes after the close of the maple sap season, their physical state observed, and the amount of paraformaldehyde remaining in the intact pellets determined. It is obvious from these data (Table 5) that the agar pellets have the highest degree of structural stability. A fairly high percentage

**TABLE 5—Physical condition of various pellet types at the end of the maple sap season, and the amount of paraformaldehyde remaining**

Paraformaldehyde pellet	Number of pellets			Average mg in intact
	Observed	Intact	Crumbled	
0.10g in 2.5% agar.....	11	11	0	4.9
0.10g in 5.0% agar.....	9	9	0	6.9
0.25g in 2.5% agar.....	12	12	0	14.0
0.25g in 5.0% agar.....	10	10	0	9.1
0.20g pressure pellet.....	12	6	6	10.0
0.40g pressure pellet.....	10	8	2	23.3
0.20g+0.2% gum arabic.....	7	3	4	11.8
0.40g+0.2% gum arabic.....	12	10	2	19.4
0.20g+1.0% gum arabic.....	10	3	7	13.8
0.40g+1.0% gum arabic.....	10	7	3	25.8

of the pressure pellets, both with and without binder, were crumbled. Thus, the influence on crumbling of added binder was not as evident as in the laboratory trials.

The amount of residual paraformaldehyde in the pellets at the end of the sap season was related to the size of the pellet (Table 5). The agar pellets with 0.25 g. paraformaldehyde had about twice the residual amount of undissolved paraformaldehyde as those containing 0.10 g.; and the pressure pellets with 0.4 g. paraformaldehyde were found to have about twice as much as the 0.2 g. pressure pellets. The paraformaldehyde remaining in the 0.2 g. pressure pellets was not significantly different from that remaining in the 0.25 g. pellets made with agar.

Shelf storage tests at room temperature were made with the various pellet types. Pellets were stored in screw cap vials (16 x 150 mm.). After 3, 6, 9, and 12 months, pellets were removed and dissolved in alkaline distilled water, and the residual paraformaldehyde determined. There was no real significant differences among pellet types in the percentage paraformaldehyde lost during shelf storage (Table 6). The loss varied from 4 to 20 percent in 3 months and from 19 to 35 percent in 6 months; but no further significant loss was observed during the last 6 months. The overall average loss from all pellets

**TABLE 6—Percent loss of paraformaldehyde during dry storage of various pellet types**

Paraformaldehyde pellet	Percent loss during storage for:			
	3 months	6 months	9 months	12 months
0.10g in 2.5% agar.....	12	24	16	20
0.10g in 5.0% agar.....	4	21	22	20
0.25g in 2.5% agar.....	20	29	29	28
0.25g in 5.0% agar.....	8	28	30	30
0.20g pressure pellet.....	12	28	18	20
0.40g pressure pellet.....	8	19	17	17
0.20g+0.2% gum arabic.....	14	35	28	26(b)
0.40g+0.2% gum arabic.....	14	22	19	20(b)
0.20g+1.0% gum arabic.....	16	33	33(a)	33(b)
0.40g+1.0% gum arabic.....	14	22	18(a)	21(b)

(a) Pellets very crumbly after 9 months storage.

(b) Pellets crumbled almost immediately after placing in water.

amounted to 24.2 percent. There was an indication that the smaller agar pellets (0.1 g.) lost a smaller percentage of formaldehyde than the larger size (0.25 g), while the reverse situation appeared to be the case with the pressure pellets either with or without binder.

The pressure pellets containing gum arabic became quite susceptible to crumbling during shelf storage. After 12 months in storage, these pellets crumbled almost instantly when put in water. Neither the pellets made with agar or the pressure pellets without binder appeared to change appreciably in this respect even during 2 years' storage.

## DISCUSSION

The results of this study reemphasize the conclusions reached by Sheneman *et al.* (5) with respect to the influence of the average temperature of maple sap seasons on the development of microorganisms in maple tree tapholes and their effect on sap yields. Thus, in very cold seasons, such as 1956, 1959, and 1960, the microorganisms have little chance to develop and any treatment used to inhibit them results in no or only small increases in sap yield. However, during warm seasons, such as 1955, 1957, and 1961, microorganisms develop rapidly in the tapholes and greatly reduce yield; an effective inhibitor will greatly increase yield.

Paraformaldehyde pellets have proven to be very effective in increasing maple sap yields during warm seasons. An increase in yield from uninoculated tappings of 96 percent was observed by Sheneman *et al.* (5) for the 1957 season; and in the current study, average increases of 17 and 62 percent were obtained for the 1959 and 1961 seasons, respectively. Only during the very cold 1960 season did paraformaldehyde treatment fail to increase yield.

It should be emphasized that the use of such an inhibitor should not and will not make the practice of good sanitation measures in sap production unnecessary. High initial microbial populations in the taphole will decrease sap yields even during extremely cold seasons (5); and, while these workers found paraformaldehyde pellets to effect complete control of even inoculated tapholes, the data presented in this study show that there is microbial development in treated tapholes late in the sap season.<sup>8</sup> The growth of microorganisms in treated

<sup>8</sup> The difference in the results is undoubtedly due to the fact that the original work was done with much larger pellets than those used in this study. Such large pellets are impractical for commercial use.

tapholes is probably more extensive than indicated in these data since the extent of development of filamentous fungi is not reflected accurately by platings made on sap flowing from the taphole. Such fungi have the greatest resistance to formaldehyde of the microorganisms involved, and the contamination of tapholes with large populations of these organisms would most certainly reduce the yield even in paraformaldehyde treated tappings. Therefore, the use of clean sanitized spiles for insertion in tapholes is necessary whether the tapholes are treated or not.

Data obtained in this study support the conclusions made by Naghski and Willits (2) that microbial activity in maple sap results in lower quality syrup. In fact in 1960, the paraformaldehyde treatment of tapholes resulted in higher quality syrup late in the season, even though the total yield was not affected. However, the paraformaldehyde treatment of tapholes is effective only in preventing high initial microbial populations in the sap as it flows from the tree by inhibiting microbial growth within the taphole, since the concentration of formaldehyde in the flowing sap is so low that it would have practically no preservative action. Thus, such treatments do not alter the necessity of keeping collection and storage containers clean and of prompt handling of sap to assure the production of high quality syrup.

Paraformaldehyde is not a permissive food additive; and, unfortunately, it is not destroyed or volatilized rapidly during the concentration of maple sap to syrup. Therefore, the use of this compound in tapholes depends on the formulation of a pellet that goes into solution at an extremely slow rate during the period that sap flows from the taphole, but results in formaldehyde levels sufficient to control microbial growth in tapholes during periods when sap is not flowing. Since the volume of sap present in the tapholes during periods of non flow is very small, the accumulated formaldehyde is diluted out rapidly.

The pellet used must also retain its structural integrity to prevent the washing out of solid paraformaldehyde with the sap. The pellets made with agar most nearly meet these requirements of the formulations studied in these experiments. Their solution rate was only about one-half that of the pressure pellets without binder; they were never observed to crumble, and the microbial control during the 1961 season must have been more complete, since the sap yield from tap-

holes treated with the agar pellets was significantly higher than from those treated with pressure pellets.

The results of this study are not too conclusive with respect to the type of agar pellet having the most desirable characteristics. There is no significant difference between pellets made with 2.5 and 5.0 percent agar, and the 1959 data failed to reveal any significant difference in yields or in control of microbial development between tapholes treated with 0.25 g. and 0.5 g. paraformaldehyde pellets made with 5 percent agar. Because of the cold 1960 season, there was no significant difference between the yields from control tapholes and from those with paraformaldehyde treatments; thus, no comparison could be made of the relative effectiveness of 0.1 g. and 0.25 g. paraformaldehyde in agar pellets which were tested that season. However, at the end of the sap season, there was an indication that the larger pellet had resulted in more complete control of microorganisms than the smaller.

These indications, plus the fact that comparable saps from tapholes treated with larger pellets consistently were higher in paraformaldehyde than from tapholes treated with smaller pellets of the same type, led the authors to conclude that the agar pellet with 0.25 g. paraformaldehyde shows the most promise of those tested. This type and size of pellet resulted in satisfactory microbial control throughout the maple season and contributed a minimum amount of formaldehyde to the sap.

The fact that small amounts of formaldehyde were found in syrups made from saps from paraformaldehyde treated tapholes means that a regulation must be issued under section 409 of the "Food Additives Amendment of 1958" before paraformaldehyde pellets should be used for treating tapholes. Such a regulation would prescribe the conditions under which paraformaldehyde may be safely used. The authors have petitioned the Food and Drug Administration to issue a food additive regulation based on the finding reported in this paper.

## SUMMARY

Representative cultures of bacteria, yeasts and molds known to cause stoppage of sap flow from maple tree tapholes were tested for their sensitivity to formaldehyde. Concentrations of about 30 p.p.m. were required to inhibit the bacteria while 100 to 150 p.p.m. were required to prevent growth of the yeasts and molds tested. The

placing of 0.1 to 0.5 g. paraformaldehyde pellets in maple tree tapholes at the beginning of the season greatly delayed the appearance of significant numbers of microorganisms in the flowing maple sap.

The effect of paraformaldehyde treatment on maple sap yield depended greatly on the average seasonal temperature. In 1959, the season was cold and paraformaldehyde increased the average yield by only 17.4 percent; 1960 was even colder and the pellets had no significant effect on the average yield; 1961, however, was a warm season and one treatment resulted in a 46 percent and the other in a 77 percent increase in yield. Two field trials were run in 1961 also; in one of these, the average increase in yield amounted to 55 percent, while in the other, it was 80 percent.

Syrups made late in the season were of higher quality when made from saps collected from treated tappings than those made from saps from comparable control tappings.

Formaldehyde concentrations were consistently low in all maple sap samples examined from treated tappings. Seventy-two percent of 901 individual sap samples examined during three seasons had less than 1 p.p.m. and only 1.2 percent contained over 10 p.p.m. No pooled lots of sap contained over 5 p.p.m. No syrups made from sap from treated tappings had residual formaldehyde levels greater than 1.6 p.p.m.; and 38 of 45 syrups made and examined had formaldehyde levels less than 1 p.p.m.

Pellets made by suspending solid paraformaldehyde in agar had slower solution rates in water and held their structural integrity longer in tapholes than did paraformaldehyde pellets made with pressure. The incorporation of either gum arabic or casein in pressure formed paraformaldehyde pellets resulted in somewhat slower solution rates but also resulted in more rapid crumbling on placing in water than similar pellets without binder.

The characteristics desired in a paraformaldehyde pellet for use in maple tree tapholes and the merits of the various pellet types tested in this study are discussed. It is concluded that pellets containing 0.25 g. paraformaldehyde, prepared by slurring paraformaldehyde in either 2.5 or 5.0 percent agar in a ratio of 1 g. per 1 ml. of melted agar has the most promise of the pellets tested in this study.

The Food and Drug Administration has been petitioned to issue a food additives regulation defining the conditions under which paraformaldehyde may be used in maple tree tapholes. The notice of Filing of Petition, dated January 11, 1962, has appeared in the Federal

Register. This petition proposes "the issuance of a regulation to establish a tolerance of 2 parts per million (0.0002 per cent) for residues of paraformaldehyde in maple syrup, from the use of paraformaldehyde in controlling microbial or fungal growth in maple tree tapholes." The petition was approved and the regulation was published in Section 121.1079 of the Federal Register, February 20, 1962.

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- (1) Upper-Peninsula Experiment Station, Chatham. Established 1907. Poultry and dairy herd management. In addition to the station proper, there is the Jim Wells Forest.
  - (2) Dunbar Forest Experiment Station, Sault Ste. Marie. Established 1925, forest management.
  - (3) Lake City Experiment Station, Lake City. Established 1928. Potatoes, breeding of beef cattle, soil and crop management.
  - (4) Graham Horticultural Experiment Station, Grand Rapids. Established 1919. Varieties, orchard soil management, spray methods.
  - (★) Michigan Agricultural Experiment Station, Headquarters, 101 Agricultural Hall, MSU, East Lansing. Established 1888. Research work in all phases of Michigan agriculture and related fields.
  - (6) Muck Experimental Farm, Laingsburg. Plots established 1941, crop production practices on organic soils.
  - (7) South Haven Experiment Station, South Haven. Established 1890. Breeding peaches, blueberries, apricots. Small fruit management.
  - (8) W. K. Kellogg Farm and Bird Sanctuary, Hickory Corners, and W. K. Kellogg Forest, Augusta. Established 1928. Forest management, milk, dairy and poultry nutrition.
  - (9) Fred Russ Forest, Cassopolis. Established 1942. Hardwood forest management.
  - (10) Ferden Farm, Chesaning. Plots established 1928. Soil management. (Land Leased)
  - (11) Streiffert Farm, Elmira. Plots established 1949. Cropping systems with special emphasis on potatoes. (Land Leased)
  - (12) Sodus Horticultural Experiment Station, Sodus. Established 1954. Production of small fruit and vegetable crops. (Land Leased)